RESEARCH

Production and Heterosis Analysis of Rice Autotetraploid Hybrids

Shengbin Tu, Li Luan, Yuhua Liu, Wenbo Long, Fanlun Kong, Tao He, Qiongfang Xu, Wengui Yan,* and Maoqun Yu*

ABSTRACT

Poor fertility is the main barrier for utilizing heterosis between the two rice (*Oryza sativa* L.) subspecies, *indica* and *japonica*. Recently, the development of autotetraploid hybrids ($2n = 4x = 48$) has been suggested as a new method for increasing heterosis in hybrid rice. Using standard experimental protocols, the elite diploid rice male sterile, maintainer, and restorer lines were colchicine-doubled, and autotetraploid counterparts were obtained. Seven resulting hybrids were analyzed for heterobeltiosis (HB), where the F_1 was compared to the male parent, and the degree of heterosis, where the F_1 was compared to the diploid commercial hybrid, Shanyou 63. The HB among the autotetraploid hybrids ranged from 1.4 to 105.9% for the productive panicles per plant, 0.5 to 74.3% for total kernels per panicle, 17.6 to 255.7% for filled kernels per panicle, and 9.6 to 130.4% for seed set. Improvements in these yield components resulted in the HB for kernel yield ranging from 64.8 to 672.7% among the seven hybrids. Hybrids T461A/T4002 and T461A/T4193 yielded 46.3 and 38.3% more, respectively, than Shanyou 63, and all other hybrids but one yielded the same or more than Shanyou 63. The high heterosis for yield suggests that hybrid sterility between two rice subspecies may be overcome by using tetraploid lines followed by intensive selection. Also, the gigantic features of the autotetraploid hybrids may establish a plant structure able to support the higher yield.

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Abbreviations: CMS, cytoplasmic male sterility; H, hybrid; HB, heterobeltiosis; IACD, immediately after chromosome doubling; M, maintainer; MS, Murashige and Skoog; R, restorer.

Heterosis, or hybrid vigor, refers to the phenomenon that progeny of diverse inbred cultivars exhibit greater biomass, faster organ development, and higher economic yield than the better of the two parents (Birchler et al., 2003; Verma and Srivastava, 2004). Heterosis utilization has been successful in many crops (Liu and Wu, 1998) but is based only on diploids in kernel crops in which the harvested parts are seeds (Cai et al., 2001; Tu et al., 2003; Yang and Virmani, 1990). Polyploid plants exist extensively in nature with the advantages of larger plant size, strong adaptive ability, and high biomass yield, thus being used in crops where the harvested parts are vegetative, such as alfalfa (*Medicago sativa* L.) (Bingham et al., 1994). High gene dosage with a variety of allelic combinations for any one gene has been proposed as an explanation of the increased productivity of polyploids (Birchler et al., 2003).

Cultivated rice (*Oryza sativa* L.) is diploid with 12 chromosome pairs ($2n = 2x = 24$, AA). Diploid rice becomes autotetraploid ($2n = 4x = 48$, AAAA) when its chromosomes are doubled (Guo et al., 2002). Similar to other crops, rice autotetraploids have

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larger seed size and weight, and increased protein and total amino acid content (Song and Zhang, 1992). The potential of using polyploids to further increase kernel yield in hybrid rice is receiving increased attention from hybrid rice breeders (Yuan, 1987). Unfortunately, partial sterility resulting in poor seed set in autotetraploid rice versus diploid rice is a major restraint to this approach (Li and Xu, 2000; Yan and Pao, 1960; Pao et al., 1985). Seed set in plants is the product of sexual reproduction. The ability for normal seed set largely depends on the reproductive processes, including pollen and embryo sac development, fertilization, embryogenesis, and endosperm development. Sterile pollen and abnormal embryo sac and fertilization have been observed in many autotetraploid rices (Guo et al., 2002; Song and Zhang, 1992; Tu et al., 2003). These abnormalities appear to be the major obstacles for normal seed set. Wang et al. (2005) observed that about two-thirds of ovaries in autotetraploid rice showed abnormal endosperm development, such as nonfertilization, irregular fertilization, endosperm development delay and nonsynchronization in development of cell walls in the aleurone layer.

Partial sterility, resulting in poor seed setting, also is a major barrier in breeding hybrid rice using *indica* and *japonica*, the two subspecies of *Oryza sativa* (Xiao et al., 1996). Seed set and kernel filling in rice hybrids between the two subspecies are not sufficiently improved to meet the requirements for commercial seed production (Wang et al., 1991), but heterosis between *indica* and *japonica* is so pronounced that these crosses have the potential to raise rice yields even higher (Liao et al., 2005; Yuan, 1987; Sun et al., 2000). This increase is important since the yields of current hybrids derived from crossing cultivars within each subspecies have reached a plateau in the past 20 yr in China (Yuan, 1987). The cytoplasmic male sterility (CMS) system plays a major role in current hybrid rice production, including male sterile, maintainer, and restorer lines, the so-called three-line system.

Li and Rutger (2007) reported heterosis for improved kernel fertility of autotetraploid rice. Breeding hybrid rice between *indica* and *japonica* would be dramatically enhanced if such fertility heterosis exists and could be incorporated into breeding programs for hybrid rice. Ploid manipulation might achieve even greater heterosis from crosses between the *indica* and *japonica* rice subspecies and result in a larger rice plant (Li and Xu, 2000; Cai et al., 2001; Huang et al., 2001), but there are no reports of breeding autotetraploid hybrid rice that approach the requirements of commercial production.

The objectives in this study were to produce autotetraploid rice plants fitting in the CMS system, including male sterile, maintainer, and restoring lines—the three-line system; improve seed set for heterosis through breeding; analyze the effects of heterosis on important agronomic characteristics; and explore the feasibility of producing autotetraploids to enhance breeding hybrids between *indica* and *japonica* in rice.

MATERIALS AND METHODS

Production of Autotetraploid Rice Lines

Elite CMS lines and their maintainer and restorer lines were bred at the Chengdu Institute of Biology, Chinese Academy of Sciences. These three types of lines were used to produce their autotetraploid counterparts by chromosome-doubling with colchicine. Young panicles at 0.5 to 2 cm long were cut into pieces and cultured in the medium containing Murashige and Skoog (MS) nutrients plus 1 mg L^{-1} 2,4-dichlorophenoxy (2,4-D), 0.2 mg L^{-1} kinetin, and 0.2 mg L^{-1} indole-3-acetic acid for callus induction. The induced calli were implanted in a secondary medium containing MS plus 0.4 mg L^{-1} vitamin B_1, 2 mg L^{-1} 2,4-D, 100 mg L^{-1} inositol, and 25 g L^{-1} mannitol, two or three times for 20 d each time. Uniformly light yellow calli with vigorous growth were selected, cut into 2-mm pieces and grown in the secondary medium plus 500 mg L^{-1} colchicine for 48 h at 25°C in shake culture. The calli were filtered with a sterile 0.5-μm screen and washed with culture fluid to remove residual colchicine and cultured in the regenerating medium containing MS plus 2 mg L^{-1} 6-benzylaminopurine, 0.5 mg L^{-1} kinetin, and 1 mg L^{-1} 1-naphthaleneacetic acid for inducing shoots. Finally, the calli with shoots were transferred to rhizogenic medium containing 0.5 MS plus 1 mg L^{-1} vitamin B_1 and 0.5 mg L^{-1} 3-indolebutyric acid for about 20 d. Plants with vigorous roots were transplanted to soil and subsequently into the field. Autotetraploids were selected based on large features, leaf color and shape, awn size, seed plumpness, or seed size (Fig. 1).

Cytological Verification of Autotetraploids

Plants growing vigorously at booting stage in the field were selected for cytological observation. After removal of old roots, the plants were cultured in liquid rhizogenic medium for 1 to 3 d until new roots grew to 1 to 2 cm. Healthy root tips were excised, treated with saturated dichlorobenzene solution for 1 to 3 h, washed three times with distilled water, and fixed in a 3:1 solution of methanol and glacial acetic acid for 24 h. The roots were then hydrolyzed with 1 mol L^{-1} HCl for 10 to 15 min at 60°C, rinsed with distilled water, and stained for 30 min using the aceto-Carmine method. The specimens were then ready for microscopic examination and photomicroscopy as described by Singh (1993).

Breeding Autotetraploid Rice Hybrids

To select for complete male sterility, autotetraploid male sterile lines were bagged to determine male sterility and crossed with the counterpart autotetraploid maintainer lines to select male maintaining ability. Male sterility and maintaining ability were enhanced with numerous test crosses and backcrosses accompanied by intensive selection. Autotetraploid restorer lines were bred for agronomic performance, male fertility or seed set, and combining ability by crossing among the autotetraploid restorer lines and test crossing with autotetraploid male sterile lines. Autotetraploid hybrids were tested for panicle fertility, kernel yield, kernel quality, and other agronomic characteristics to

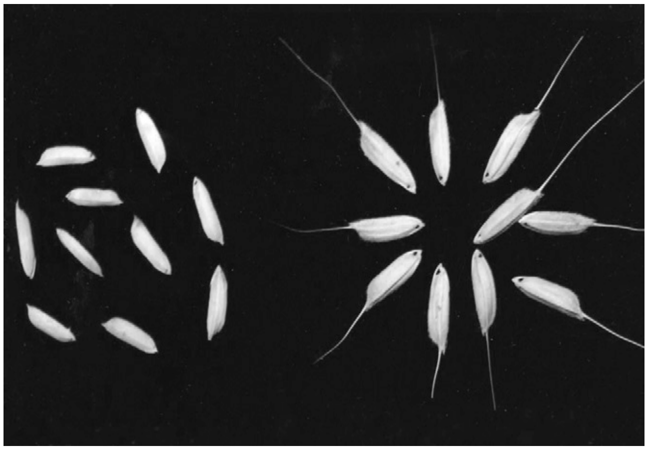

Figure 1. Autotetraploid Minghui 63 (right) induced by colchicines from diploid Minghui 63 calli had larger kernels with awns in various lengths as compared to its diploid parent Minghui 63 (left), commercial restorer line in the hybrid rice of cytoplasmic male sterile system.

identify a good combination of autotetraploid male sterile lines, maintainer lines and restorer lines for hybrid rice breeding.

Table 1. Autotetraploid rice male sterile lines, maintainer (M) lines, restorer (R) lines, and F_1 hybrids (H) in cytoplasmic male sterile (CMS) system induced from diploid counterparts by doubling chromosomes with colchicine.

Designation	Origin†	Status in CMS system	Ploidy	Verified chromosomes
T461A	CIB.CAS	Male sterile line	4*x*	$2n = 4x = 48$
T462A	CIB.CAS	Male sterile line	4*x*	$2n = 4x = 48$
T402A	CIB.CAS	Male sterile line	4*x*	$2n = 4x = 48$
Gang 46A (check)	RRI.SAU	Male sterile line	2*x*	$2n = 2x = 24$
T446B	CIB.CAS	M line	4*x*	$2n = 4x = 48$
T402B	CIB.CAS	M line	4*x*	$2n = 4x = 48$
T419B	CIB.CAS	M line	4*x*	$2n = 4x = 48$
Gang 46B (check)	RRI.SAU	M line	2*x*	$2n = 2x = 24$
T4509	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4002	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4043	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4237	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4001	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4039	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4063	CIB.CAS	R line	4*x*	$2n = 4x = 48$
T4193	CIB.CAS	R line	4*x*	$2n = 4x = 48$
Minghuo 63 (check)	Fujian	R line	2*x*	$2n = 2x = 24$
T462A/T4509	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T461A/T4002	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T461A/T4039	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T462A/T4043	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T461A/T4237	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T461A/T4193	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
T461A/T4063	CIB.CAS	F_1 H	4*x*	$2n = 4x = 48$
Shanyou 63 (check)	Sichuan	F_1 H	2*x*	$2n = 2x = 24$

†CIB.CAS, Chengdu Institute of Biology, Chinese Academy of Sciences; RRI.SAU, Rice Research Institute, Sichuan Agricultural University; Fijian, Fujian Province, China; Sichuan, Sichuan Province, China.

Field Experiments

Seed of three autotetraploid CMS lines with the diploid MS check Gang 46A, three maintainer (M) autotetraploid lines with the diploid M check Gang 46B, eight restorer (R) autotetraploid lines with the diploid R check Minghui 63, and seven resulting F_1 hybrids (H) with the diploid H check Shanyou 63 were prepared in summer 2002 at the experimental farm of the Chengdu Institute of Biology, Chinese Academy of Sciences in Wenjiang, Sichuan Province, China (Table 1). Diploid Gang 46A, Gang 46B, Minghui 63, and Shanyou 63 are commercial CMS, M, R, and H lines, respectively, and are widely grown in China. In winter 2002, each entry was planted in a 2- × 1-m plot in Lingshui, Hainan, China. Sixty plant hills in each plot were arranged in five rows at a density of 300,000 plants per hectare. In March 2003, three plants from each row, or 15 plants from each entry, were randomly sampled for data collection at maturity. Nine agronomically important characteristics were recorded: plant height (cm) from ground to panicle tip, productive panicles as the number of panicles having five or more fully matured kernels per plant, panicle length (cm), total kernels per panicle, filled kernels per panicle, seed set (%), flag leaf length and width (cm), kernel length and width (mm), 1000-kernel weight (g) and yield (t ha^{-1}) estimated as total kernel weight per plant (g) multiplied by 0.3. Heterosis in F_1 hybrids (Matzinger et al., 1962) was estimated as heterobeltiosis for comparison of F_1 with the better parent (Fonseca and Patterson, 1968), and competitive or standard heterosis for comparison of F_1 with the best commercial cultivar (Virmani et al., 1982). In the present study, the heterobeltiosis was calculated using comparison with the male parent or R line, since the female parent was male sterile. The competitive heterosis was calculated using comparison with Shanyou 63, a commercial hybrid used as a check in National Rice Uniform Test in China. The data were analyzed by SAS statistical software, version 9.1.3 (SAS Institute, 2004).

RESULTS

Production of Autotetraploid Male Sterile Lines, Maintainer Lines, Restorer Lines, and F_1 Hybrids

Autotetraploid Minghui 63 induced by colchicines-doubling from diploid Minghui 63 calli had larger kernels with awns in various lengths compared with its diploid parent Minghui 63, the commercial R line in the hybrid rice of cytoplasmic male sterile system (Fig. 1). Awns and larger kernels were usually observed in autotetraploid rice, providing morphological proof of chromosome doubling (Fig. 1). Three autotetraploid male sterile lines, three M lines and eight R lines were developed (Table 1). The seven hybrid combinations made from these male sterile and R lines had agronomic value. Autotetraploid was documented with photomicrographs of the 48 chromosomes (Fig. 2, Table 1), and a field experiment compared these autotetraploid lines with the diploid Chinese commercial checks, male sterile line Gang 46A, M line Gang 46B, R line Minhui 63, and H Shanyou 63.

Comparisons of Autotetraploid Cytoplasmic Male Sterile, Maintainer, and Restorer Lines and Hybrids with Their Diploid Counterparts for Agronomically Important Characteristics

Male Sterile Lines

Among the three autotetraploid CMS lines, two had taller plants, more productive panicles and longer panicles than diploid MS line Gang 46A (Table 2). A dramatic decrease of total kernels in a panicle but an increase in flag leaf length and width were observed in all three MS lines compared with Gang 46A.

Maintainer Lines

All three autotetraploid M lines were taller with longer panicles, longer and wider flag leaves, and longer and heavier kernels than diploid M line Gang 46B (Table 2). T446B and T402B had more total kernels and filled kernels per panicle, greater seed set and higher yield, and the same number of productive panicles compared with Gang 46B. Autotetraploid T419B had fewer productive panicles per plant than the other two autotetraploid M lines and diploid Gang 46B.

Restorer Lines

Comparing the eight autotetraploid R lines to the diploid check, Minghui 63, the autotetraploid R lines had kernels that were 5.0 to 30.8 g per 1000 kernels heavier, 0.5 to 2.1 mm longer, and 0.1 to 1.1 mm wider; flag leaves that were 0.8 to 17.9 cm longer and 0.3 to 1.3 mm wider; and panicles that were 1.3 to 8.9 cm longer. But there were 4.0 to 9.1 fewer productive panicles per plant and 9.0 to 76.6 fewer filled kernels per panicle (Table 2). Among the autotetraploid R lines, there was much variation for productive panicles, filled kernels per panicle, and kernel length and weight; however, T4001 had the most productive panicles and the second most filled kernels and kernel length.

The seed weight of T4237 was much greater than the other lines. Among eight autotetraploid R lines, total kernels per panicle varied from 73.6 to 173.2, with T4001 and T4193 having 48.9 and 28.3 more total kernels, respectively, than Minghui 63. Two R lines (T4043, T4237) had total kernels similar to Minghui 63, but four others had 13.6 to 50.7 fewer total kernels than the check. Four R lines were taller, one was shorter, and three had the same plant height as Minghui 63. The yields of eight R lines were 2.6 to 10.0 t ha^{-1} less than diploid Minghui 63 (11.1 t ha^{-1}).

F_1 Hybrids

Compared with diploid hybrid Shanyou 63, all seven autotetraploid hybrids had kernels that were 8.1 to 29.9 g per 1000 kernels heavier, 1.5 to 2.8 mm longer, and 0.1 to 0.7 mm wider and flag leaves 4.7 to 17.0 cm longer and 0.1 to 0.6 cm wider. But there were 2.9 to 7.3 fewer productive panicles per plant. All but one autotetraploid hybrid was taller and had longer panicles than Shanyou 63, and only

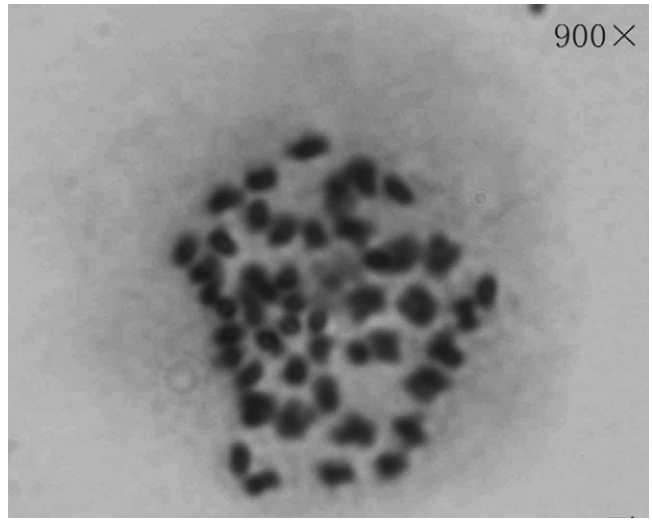

Figure 2. Cytological examination of chromosome performance in the autotetraploid rice induced by colchicines from diploid calli showed $2n = 4x = 48$ chromosomes.

T461A/T4002 had more total kernels per panicle than Shanyou 63. Four hybrids had 13.5 to 39.7 fewer total kernels, three had more filled kernels per panicle, and four had fewer filled kernels per panicle than Shanyou 63. The kernel fertility of autotetraploid hybrids was significantly improved, with three hybrids having 13.5 to 17.8% more seed set than Shanyou 63 and three having the same seed set. This resulted in four hybrids with yields that were 1.5 to 4.4 t ha^{-1} more than Shanyou 63.

Heterobeltiosis and Competitive Heterosis for Agronomically Important Characteristics among Seven Autotetraploid Hybrids

Heterobeltiosis

Positive heterobeltiosis or increased effects were observed for all characteristics related to kernel fertility and yield, that is, productive panicles per plant, total kernels and filled kernels per panicle, seed set percentage, kernel length and width, 1000-kernel weight, and yield (Table 3) when seven autotetraploid hybrids were compared with their respective male parent or R line. Yield increased by 302.8%, ranging from 64.8 to 672.7%, filled kernels per panicle increased by 110.6% on average, and kernel length increased by 3.9%. The other two yield components, productive panicles per plant and percent seed set had positive heterobeltiosis averaging more than 50% and ranging from 1.4 to 105.9% and 9.6 to 130.4%, respectively. Negative heterobeltiosis, or decreased effect, was observed in two hybrids for plant height, four hybrids for flag leaf length, two hybrids for flag leaf width, and two hybrids for panicle length.

Competitive Heterosis

Positive competitive heterosis or increased effects were observed for flag leaf length and width, panicle length, kernel length and width, and 1000-kernel weight (Table

Table 2. Agronomically important characteristics of autotetraploid (4*x*) cytoplasmic male sterile (CMS) lines, maintainer (M) lines, restorer (R) lines and F_1 hybrids (H) in comparison with their diploid (2*x*) counterparts in rice.

Designation	Ploidy, status	Plant height	Productive panicles plant^{-1}	Panicle length	Total kernels panicle^{-1}	Filled kernels panicle^{-1}	Seed set Mean	Seed set Max.	Flag leaf length	Flag leaf width	Kernel length	Kernel width	1000-kernels	Yield
		cm		cm			— % —		— cm —		— mm —		g	t ha^{-1}
T461A	4*x*, CMS	76.8	14.9	17.9	102.7				25.1	2.1				
T462A	4*x*, CMS	84.0	20.3	19.1	106.5				25.0	2.1				
T402A	4*x*, CMS	73.7	17.7	23.8	121.2				26.1	2.0				
Gang 46A (check)	2*x*, CMS	72.4	15.1	18.1	136.5				21.0	1.5				
T446B	4*x*, M	82.3	14.2	18.4	109.8	96.7	88.1	88.9	25.0	2.1	9.1	3.0	31.4	10.3
T402B	4*x*, M	77.7	13.6	22.1	115.0	80.6	70.1	78.2	27.2	2.0	9.2	3.4	32.8	8.8
T419B	4*x*, M	97.0	6.0	22.7	113.9	69.4	60.9	68.0	30.7	1.8	10.2	3.1	37.0	3.7
Gang 46B (check)	2*x*, M	64.1	14.2	17.0	104.0	67.8	65.2	69.1	21.6	1.5	8.8	3.0	27.0	6.4
T4509	4*x*, R	94.8	8.2	20.1	110.7	84.3	76.2	87.6	29.7	1.8	9.5	3.4	33.3	5.5
T4002	4*x*, R	98.2	4.9	19.2	96.7	58.8	62.1	62.8	34.8	2.6	10.3	3.8	33.4	2.4
T4043	4*x*, R	81.7	6.0	21.5	120.8	74.1	61.4	75.2	24.4	1.7	10.2	3.1	33.4	3.5
T4237	4*x*, R	96.4	7.0	21.0	120.0	72.5	60.4	78.7	27.7	2.0	11.0	4.1	58.0	7.1
T4039	4*x*, R	83.9	7.2	26.0	73.6	27.3	39.2	37.0	28.1	1.6	9.8	3.3	32.2	1.5
T4063	4*x*, R	83.9	5.1	25.8	76.6	26.4	34.5	51.3	27.1	1.7	9.4	3.5	32.6	1.1
T4193	4*x*, R	111.5	5.7	26.8	152.6	94.0	61.6	77.9	40.1	2.1	10.6	3.9	37.5	4.8
T4001	4*x*, R	85.8	10.0	22.7	173.2	88.7	51.2	56.4	23.0	1.6	10.9	3.8	39.9	8.5
Minghui 63 (check)	2*x*, R	85.8	14.0	17.9	124.3	103.0	82.9	91.1	22.2	1.3	8.9	3.0	27.2	11.1
T462A/T4509	4*x*, H	136.5	10.9	25.8	111.3	103.7	93.1	96.9	37.7	1.8	9.8	3.5	40.3	10.9
T461A/T4002	4*x*, H	104.4	9.3	26.0	168.5	150.2	89.1	92.0	33.3	2.1	11.0	3.9	41.2	13.8
T461A/T4039	4*x*, H	93.6	10.0	22.0	125.2	97.1	77.5	88.9	26.1	1.9	10.1	3.7	39.1	9.1
T462A/T4043	4*x*, H	113.9	9.0	24.8	140.9	94.9	67.3	85.0	38.0	2.2	10.9	3.8	40.0	8.1
T461A/T4237	4*x*, H	97.7	6.9	29.3	135.4	120.1	88.7	90.2	29.4	2.1	11.0	4.2	59.1	11.7
T461A/T4193	4*x*, H	93.3	11.3	26.6	157.6	120.5	76.5	96.7	35.9	1.7	11.0	4.0	40.0	13.0
T461A/T4063	4*x*, H	80.3	10.9	20.4	109.2	86.8	79.5	87.9	25.7	1.9	9.7	3.6	37.3	8.5
Shanyou 63 (check)	2*x*, H	86.4	14.2	20.1	148.9	112.0	75.2	95.1	21.0	1.6	8.2	3.5	29.2	9.4
LSD 0.05		2.1	1.3	0.7	9.2	4.7	5.2		0.8	0.1	0.1	0.1	1.5	1.1
CV (%)		3.2	17.0	5.0	11.0	7.5	10.4		3.7	4.4	1.0	2.6	5.7	20.5

3) when the seven autotetraploids were compared with the best diploid commercial hybrid cultivar, Shanyou 63. Flag leaf length increased by an average of 53.8% (range 22.4–79.5%), and 1000-kernel weight increased by 45.3% (range 27.7–102.4%) among the seven hybrids. But the productive panicles per plant among the seven hybrids averaged −31.3% with a range of −51.4 to –20.4% among the seven hybrids compared with Shanyou 63. Six of seven autotetraploid hybrids increased in height and seed set, three out of the seven increased in total kernels and filled kernels per panicle, and four increased in yield over Shanyou 63.

Heterobeltiosis and Competitive Heterosis for Autotetraploid Hybrids on Agronomically Important Characteristics

Heterobeltiosis

Three hybrids, T462A/T4509, T462A/T4043, and T461A/T4237, had positive heterobeltiosis or increase effects over their respective R lines for all 12 characteristics tested in this study (Table 4). Two hybrids, T461AXT4002 and T461AXT4039, had positive heterobeltiosis for 10 of 12 traits. Most positive heterobeltiosis or gains over their respective R lines were observed for productive panicles per plant in T461A/T4063 at 105.9%; total kernels per panicle in T461A/T4002 at 74.3%; filled kernels per panicle in T461A/T4039 at 255.7%; seed set in T461A/T4063 at 130.4%; yield in T461A/T4063 at 672.7%; and kernel weight in T461A/T4002 at 23.4%. Three hybrids, T461A/T4002, T461A/T4039, and T461A/T4063, had positive heterobeltiosis of about 500% or more for yield.

Competitive Heterosis

Two hybrids, T461A/T4002 and T461A/T4237, had positive competitive heterosis or increased effects over the diploid hybrid Shanyou 63 for 11 of 12 characteristics tested in this study (Table 4). The one exception was productive

Table 3. Analysis of heterobeltiosis and competitive heterosis among seven autotetraploid F_1 hybrids in cytoplasmic male sterile system on agronomically important characteristics in rice.

Traits	Heterobeltiosis [(F_1–Restorer/Restorer)100%]				Competitive heterosis [(F_1–Shanyou 63/Shanyou 63)100%]			
	Hybrids with positive and negative effect†	Minimum	Maximum	Mean	Hybrids with positive and negative effect	Minimum	Maximum	Mean
		——— % ———				——— % ———		
Plant height	5 (2)	–16.3	44.0	11.7	6 (1)	–7.1	58.0	19.0
Flag leaf length	3 (4)	–10.5	55.7	8.8	7 (0)	22.4	79.5	53.8
Flag leaf width	5 (2)	–19.2	29.4	3.8	7 (0)	6.2	37.5	22.3
Productive panicles plant^{-1}	7 (0)	1.4	105.9	59.6	0 (7)	–51.4	–20.4	–31.3
Panicle length	5 (2)	–20.9	39.5	11.7	7 (0)	1.5	45.8	24.3
Total kernels panicle^{-1}	7 (0)	0.5	74.3	31.5	3 (4)	–26.7	13.2	–9.0
Filled kernels panicle^{-1}	7 (0)	17.6	255.7	110.6	3 (4)	–15.3	34.1	–1.4
Seed set	7 (0)	9.6	130.4	55.2	6 (1)	–10.5	118.0	22.9
Kernel length	7 (0)	0.0	6.9	3.9	7 (0)	18.3	34.1	28.0
Kernel width	7 (0)	2.4	22.6	6.9	7 (0)	0.0	20.0	9.0
1000-kernel weight	7 (0)	1.9	23.4	15.5	7 (0)	27.7	102.4	45.3
Yield	7 (0)	64.8	672.7	302.8	4 (3)	–13.8	159.6	16.9

†Number of hybrids with negative effect is listed in the parentheses.

panicles per plant. Three hybrids, T462A/T4509, T461A/T4193, and T461A/T4063, showed the least decrease in competitive heterosis for productive panicles at −21.4% compared with Shanyou 63. The most advantages over Shanyou 63 were observed in T461A/T4002 with 36.3% for total kernels per panicle and 45.6% for filled kernels per panicle, which resulted in the highest competitive heterosis for yield at 23.6% among seven hybrids. Hybrid T462A/T4509 had the most competitive heterosis for seed set with 12.3%, and T461A/T4237 had the most for kernel weight with 117.3%.

Correlations of the Characteristics Associated with Kernel Fertility

Grain yield resulting from yield components highly correlated with filled kernels per panicle (r = 0.9), seed set percentage (r = 0.8), total kernels per panicle (r = 0.7), and productive panicles per plant (r = 0.6). Filled kernels per panicle correlated with total kernels per panicle (r = 0.9), and seed set percentage (r = 0.8).

Table 4. Comparative analysis of agronomically important characteristics in rice between heterobeltiosis and competitive heterosis for each autotetraploid F_1 hybrid in cytoplasmic male sterile system.

Hybrid combination	Heterosis type	Plant height	Productive panicles/ plant	Panicle length	Total kernels/ panicle	Filled kernels/ panicle	Seed set	Flag leaves		Kernels		1000-kernels	Yield
								length	width	length	width		
		cm		cm			%	— cm —		— mm —		g	t ha^{-1}
T462A/T4509	Heterobeltiosis (%)	44.0	33.0	28.4	0.5	23.7	22.2	26.9	0.0	3.2	2.9	21.0	98.2
	Competitive heterosis (%)	59.1	–21.4	36.5	–10.5	1.0	12.3	69.8	38.5	10.1	16.7	48.2	16.0
T461A/T4002	Heterobeltiosis (%)	6.3	89.8	35.4	74.3	155.6	43.5	–4.3	–19.2	6.8	2.6	23.4	475.0
	Competitive heterosis (%)	21.7	–35.7	37.6	36.3	45.6	7.5	50.0	61.5	23.6	30.0	51.5	46.8
T461A/T4039	Heterobeltiosis (%)	11.6	38.9	–15.4	70.1	255.7	109.5	–7.1	18.8	3.1	12.1	21.4	506.7
	Competitive heterosis (%)	9.1	–28.6	16.4	0.8	–5.8	–6.5	17.6	46.2	13.5	23.3	43.8	–3.2
T462A/T4043	Heterobeltiosis (%)	39.4	50.0	15.3	16.6	28.1	9.6	55.7	29.4	6.9	22.6	19.8	131.4
	Competitive heterosis (%)	32.8	–35.7	31.2	13.7	–7.8	–18.8	71.2	69.2	22.5	26.7	47.1	–13.8
T461A/T4237	Heterobeltiosis (%)	1.3	1.43	39.5	12.8	65.7	46.9	6.1	5.0	0.0	2.4	1.9	64.8
	Competitive heterosis (%)	13.9	–50.0	55.0	8.9	16.5	7.0	32.4	61.5	23.6	40.0	117.3	24.5
T461A/T4193	Heterobeltiosis (%)	–16.3	98.3	–0.7	3.3	17.6	24.2	–10.5	–19	3.8	2.6	6.7	170.8
	Competitive heterosis (%)	8.7	–21.4	40.7	27.4	17.5	–7.7	61.7	30.8	23.6	33.3	47.1	38.3
T461A/T4063	Heterobeltiosis (%)	–4.3	105.9	–20.9	42.6	228.8	130.4	–5.2	11.8	3.2	2.9	14.4	672.7
	Competitive heterosis (%)	–6.4	–21.4	7.9	–12.1	–15.5	–4.1	15.8	46.2	9.0	20.0	37.1	–9.6

DISCUSSION

Breeding Autotetraploid Hybrid Rice

In general, rice autotetraploids have poor seed set, kernel filling, and other characteristics of agronomic importance (Li and Xu, 2000; Yan and Pao, 1960; Pao et al., 1985). This is especially true in the first generation of autotetraploid after chromosome doubling where very significant reductions are commonly observed for seed set, total kernels per panicle, tillers per plant, and panicle exsertion compared with the diploid rice parents. Noteworthy variations in these characteristics were identified among different panicles in a plant, among different plants in an autotetraploid line, among different generations of the same line, and among different lines, which present potential for improving these traits. Male sterile plants were pollinated immediately after chromosome doubling (IACD) with autotetraploid maintainer plants in advanced generations after the doubling to initiate breeding for autotetraploid male sterile lines. Crossing the IACD restorer plants with advanced or bred R lines and crossing the IACD maintainer plants with advanced or bred M plants, along with test-crossing, were done to breed autotetraploid male R lines and M lines. More than 100 F_1 hybrids have been produced and tested in the past 12 yr as part of the Chengdu Institute of Biology, Chinese Academy of Sciences hybrid rice breeding program. A set of elite autotetraploid male sterile lines (T461A and T462A), their counterpart maintainers with high seed set, and R lines with high combining abilities (T4002 and T4193) were developed with significantly improved kernel fertility, kernel weight, and agronomic characteristics (plant height, tillering, panicle size, and awn type). Seed sets of autotetraploid R lines T4002, T4509, and T4043 and M line T461B increased from 47.4, 33.6, 32.1, and 21.2% in 1996 to 59.4, 63.4, 48.7, and 62.2% in 2000, respectively, by selecting for improved kernel type, agronomic characteristics, and seed set. These improvements suggest that kernel fertility of autotetraploid M and R lines in the autotetraploid CMS system could be sufficiently improved for commercial production of hybrid rice seed.

Heterosis of Kernel Fertility in Autotetraploid Hybrid Rice

Hybrid sterility is common in crosses between *indica* and *japonica* rice (Sano, 1993; Wan et al., 1996; Wan and Ikehashi, 1997), but in the present study, heterosis for kernel fertility was high in autotetraploid hybrids between completely sterile female parents and male parents with varying sterility levels (Table 2). Seed set varied from 34.5 to 76.2% with an average of 55.8% in the eight autotetraploid R parents, which was 27.1% less than the diploid check, Minghui 63. Seed set in seven autotetraploid hybrids resulting from these parents ranged from 67.3 to 93.1% with an average of 81.7%, which was 6.5% more than that in the diploid check, Shanyou 63. All the hybrids had higher seed set, ranging from 5.9 for T462A/T4043 to 45.0% for T461A/T4063, than their respective male parent or R line. Heterosis in all but one hybrid for kernel fertility resulted in an equal or greater seed set compared with Shanyou 63 (Table 2). High heterosis for kernel fertility also was identified in autotetraploid rice developed in the United States (Li and Rutger, 2007), suggesting that autotetraploids possibly could be used to develop hybrids between *indica* and *japonica* rice.

In addition to improved seed set, these autotetraploid hybrids had a heterobeltiosis for productive panicles per plant ranging from 1.4 to 105.9%, total kernels per panicle from 0.5 to 74.3%, and filled kernels per panicle from 17.6 to 255.7%, which affected kernel yield. The heterobeltiosis for yield of the hybrids over their male parents ranged from 64.8 to 672.7%, which is much greater than the value of 59% reported for diploid hybrids (Virmani et al., 1982), signifying an advantage of autotetraploid hybrids over pure line autotetraploid rice cultivars. Also, the huge difference of heterobeltiosis for yield and yield components between diploid and autotetraploid hybrids indicates that this methodology should be exploited further.

Autotetraploid plants have poorer fertility but greater crossing ability than diploid plants (Huang, 2000; Huang et al., 2001; Song and Zhang, 1992). This is evidenced by the fact that Huang (2000, 2001) successfully hybridized *Pennisetum alopecuroides* and *Leersia hexandra* with autotetraploid rice but not with diploid rice. Also, autotetraploid hybrids had fewer abnormalities during meiosis than autotetraploid pure lines, including fewer univalents and chromosomes delayed during division (Tu et al., 2003). Huang et al. (2004) reported that hybrid sterility in diploid hybrids between *indica* and *japonica* could be overcome at the tetraploid level, and that autotetraploid hybrids between *indica* and *japonica* reached normal fertility. The improved heterosis for yield and its components revealed in this study verifies previous research and suggests a method for dealing with hybrid sterility and using hybrid vigor between the two rice subspecies.

Commercial Potential of Autotetraploid Hybrid Rice

The gigantic features of autotetraploid hybrids were observed in the root system, stems, leaves, panicles, and kernel size (Huang et al., 2001; Song and Zhang, 1992; Birchler et al., 2003). In rice the enlarged stems increased straw strength, made the plant erect, and reduced lodging, which enabled the plant to support more seeds per panicles, thus identifying an ideal plant type for a higher-yielding rice plant. Also, in alfalfa (*Medicago sativa* L.) the stress resistance of tetraploid exceeds that of diploid (Bingham et al., 1994). In our study, the yield of all but one hybrid was the same or more than Shanyou 63, the best Chinese hybrid cultivar.

In conclusion, hybrid T462AXT4509 had a yield of 13.9 t ha^{-1} in 2002 and 13.3 t ha^{-1} in 2003, which was

52.2% more than 'D-You 527' (9.13 t ha^{-1} in 2002), the most recently released rice hybrid for southwest China (Tu et al., 2003). This indicates that some autotetraploid hybrid combinations are approaching commercial yield levels. Improved kernel yield depends on increases in filled kernels per panicle, percent seed set, total kernels per panicle, and productive panicles per plant. As a result of this study, current efforts are focused on improving seed production in the hybrids and reproducing male sterile lines, in addition to improving the traditional agronomic, kernel, and milling-quality characteristics.

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